Data Evaluation Report on the Acute Toxicity of 2,4-DB DMAS to the Freshwater Diatom *Navicula pelliculosa*

DP Barcode: D439488 MRID No.: 49833102

Data Requirement:	EPA PC Codes	030801 & 030819
	EPA DP Barcode	439488
	EPA MRID	49833102
	EPA Guideline	850.4500

Test material: 2,4-DB DMAS

Purity: 500 g/L 2,4-DB DMAS (43.78% w/w 2,4-DB)

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CITATION: Amoroso, T. 2016. 2,4-DB DMAS: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*. Unpublished study performed by ABC Laboratories, Inc., Colombia, Missouri. Laboratory Study No. 82627. Study sponsored by the 2,4-DB Task Force c/o Data Group Management, Raleigh, North Carolina. Study initiated August 28, 2015 and completed January 29, 2016.

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater diatom *Navicula pelliculosa* (strain not reported) were exposed to 2,4-DB DMAS (43.78% w/w 2,4-DB) at nominal concentrations of 0 (negative control), 0.94, 1.9, 3.8, 7.5, 15, and 30 mg ae/L under static conditions. Mean-measured concentrations were <0.041 (<MQL, negative control), 1.03, 2.12, 4.21, 8.47, 17.0, and 32.8 mg ae/L, representing 109, 111.6, 110.7, 112.9, 113.3, and 109.2% of nominal, respectively.

After 96 hours, the most sensitive endpoint was area under the growth curve (AUC) with an IC_{50} value of 9.38 mg ae/L, and NOAEC and IC_{50} values of 4.21 and 9.38 mg ae/L (mean-measured concentrations), respectively. NOAEC and IC_{50} values for yield were 4.21 and 9.44 mg ae/L, respectively, and NOAEC and IC_{50} values for growth rate were 4.21 and 20.0 mg ae/L, respectively. The reported percentage growth inhibition in the treated algal culture as compared to the control ranged from -2 to 96%.

This study **is scientifically sound** and is classified as **acceptable**.

Results Synopsis

Yield

IC_{05}: 4.96 mg ae/L	95% C.I.: 4.62-5.24 mg ae/L
IC_{50}: 9.44 mg ae/L	95% C.I.: 9.24-9.64 mg ae/L
NOAEC: 4.21 mg ae/L	LOAEC: 7.5 mg ae/L

Growth rate

IC_{05}: 4.47 mg ae/L	95% C.I.: 3.08-5.56 mg ae/L
IC_{50}: 19.99 mg ae/L	95% C.I.: 18.26-21.88 mg ae/L
NOAEC: 4.21 mg ae/L	LOAEC: 7.5 mg ae/L

Area under the curve (AUC)

IC_{05}: 3.9 mg ae/L*	95% C.I.: 3.34-4.32 mg ae/L
IC_{50}: 9.38 mg ae/L	95% C.I.: 8.97-9.8 mg ae/L
NOAEC: 4.21 mg ae/L	LOAEC: 7.5 mg ae/L

Note that the IC_{05} value for area under the growth curve is below the estimated NOAEC value, but that the NOAEC falls within the 95% CI for the estimated IC_{05} value. This endpoint should only be used with caution.

Endpoint(s) Affected: Yield, growth rate, and area under the curve
Most Sensitive Endpoint: Area under the curve

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on U.S. Environmental Protection Agency Ecological Effects Test Guidelines, OCSPP Number 850.4500: *Algal Toxicity* (2012). The following deviations from U.S. EPA OCSPP 850.4500 guideline were noted:

1. The strain of the test organism was not reported.
2. The health/condition of the inoculum culture was not described; the inoculum should be from a logarithmically growing stock culture.
3. The study author did not report the hardness, alkalinity, pH, specific conductivity, total organic carbon, COD, and particulate matter of the dilution water, as required by OCSPP guidance.

These deviations **do not** affect the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided. The study was conducted in compliance with the GLP standards of U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 1989) with the exception of the water characterizations performed in June 2015. This exception did not adversely affect the study integrity or the interpretation of the study results.

A. MATERIALS:

1. Test material	2,4-DB DMAS
Description:	Pale brown liquid
Lot No./Batch No. :	JPB/596/046
Purity:	500 g/L 2,4-DB DMA (43.78% w/w 2,4-DB)

Stability of compound

under test conditions: 96-hour measured concentrations ranged from 94 to 102% of their 0-hour measured counterparts. The 96-hour measured concentration of the abiotic control at the 0.94 mg/L concentration was 1.08 mg/L, whereas the concentration its biotic counterpart was 1.01 mg/L, indicating that the presence of the algal biomass did not impact the stability of the test substance under test conditions.

(OECD recommends stability in water and light)

Storage conditions of test chemicals: Room temperature

2. Test organism:

Name: Freshwater diatom, *Navicula pelliculosa*

This test is conducted with a nonvascular species, including at a minimum the freshwater alga P. subcapitata (formerly S. capricornutum), freshwater diatom N. pelliculosa, and the marine diatom S. costatum. Other test species may need modification of the test method. For Tier I studies, only the freshwater alga P. subcapitata is recommended. The cyanobacterium A. flos-aquae test is found in EPA guideline 850.4550. OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported.

Strain: Not reported

Source: In-house cultures originally obtained from the Department of Botany, Culture Collection of Algae, University of Texas at Austin

EPA recommends algae be from the same source and stock culture or commercial sources.

Age of inoculum: 3 days

EPA recommends the algal inoculum should be from logarithmically growing stock cultures (typically 3- to 7-days old).

Method of cultivation: Cultured in freshwater algal nutrient medium with sodium silicate (FWAM = Si) under continuous cool-white fluorescent light (4109 to 4736 lux) at 24 ± 2ºC and shaken at 100 rpm.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Two range-finding toxicity tests were conducted in September 2015 to determine definitive test concentrations. In the first test, percent inhibition in cell density (as compared to the negative control response) at 96 hours was 16, 24, 19, 14, 41, and 99% in the nominal 0.0010, 0.010, 0.10, 1.0, 10, and 100 mg ae/L groups, respectively. Atypical growth was observed in the controls, and variable cell densities were observed in all first range-finding test levels. The growth inhibition observed in the two highest concentrations provided the upper range of definitive test concentrations, but a second range-finding test was conducted to more closely define the lowest target concentration. In the second test, percent inhibition in cell density (as compared to the negative control response) at 96 hours was -3, -1, -2, -1, -1, and 24% in the nominal 0.00010, 0.0010, 0.010, 0.10, 1.0, and 5.0 mg ae/L groups, respectively. Based on these results, the definitive study was conducted using nominal concentrations of 0 (control), 0.94, 1.9, 3.8, 7.5, 15, and 30 mg ae/L.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	**Details**	**Remarks**
		Criteria
Acclimation period:	Continuously cultured in-house	None.
Culturing media and conditions: (same as test or not)	Same as test	*EPA recommends the algal inoculum used to initiate toxicity testing is from a liquid culture shown to be actively growing (i.e. capable of logarithmic growth within the test period) in at least two subcultures lasting 7 days each prior to the start of the definitive test. A culture should not be used if it is contaminated by fungi/other algae or if test algae were used in a previous test.*
Health: (any mortality observed)	Not reported	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*

Parameter	**Details**	**Remarks**
		Criteria
Test system: Static/static renewal Renewal rate for static renewal	Static N/A	None *EPA recommends a static exposure technique. Although semi-continuous algal culturing techniques are available, they have not been commonly employed in algal toxicity testing and their use is not recommended.*
Incubation facility:	Test vessels were maintained in an environmental chamber.	N/A
Duration of the test:	96 hours	None *EPA recommends 96 hours at a minimum.* *OECD: 72 hours.*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Not reported 250 mL 100 mL	Erlenmeyer flasks were plugged with foam stoppers. *EPA recommends 125-500 mL Erlenmeyer flasks and test solution volume ≤50% of flask volume. Flasks may be covered with foam plugs (that are proven non-toxic), stainless steel caps, Shimadzu enclosures, glass caps or screw caps. EPA recommends all test vessels and closures to be identical. OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

Parameter	**Details**	**Remarks**
		Criteria
Details of growth medium Name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	Freshwater algal medium with sodium silicate (FWAM+Si). 7.4 to 7.5 7.8 to 8.5 $Na_2EDTA•2H_2O$ $NaHCO_3$ N/A	The medium was prepared by the addition of reagent grade salts to autoclaved ABC reagent water. The pH was adjusted to 7.5 ± 0.1 with 0.1 N NaOH and 0.1 N HCl, and medium filtered through 0.2-µm Millipore® filters.
		EPA recommends an AAP medium with chelating agents (e.g. EDTA) prepared according to EPA's 850.4500 guideline (http://www2.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines). Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium for test solution preparation if it is suspected that the chelator will interact with the test material. *EPA recommends adjustment of pH before adding inoculum, if pH of test solution is <5 or highly basic.* *OECD recommends the medium pH after equilibration with air is ~8 with less than 0.001 mmol/L of chelator if used.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	A standard nutrient medium was used and a detailed description was provided.	

Parameter	**Details**	**Remarks**
		Criteria
<u>Dilution water used to prepare media</u> Source: <u>Quality</u> Hardness: Alkalinity: pH: Specific conductivity: Salinity (**for marine algae**): Water pretreatment (if any): TOC: COD: Particulate matter: Metals: Pesticides/PCBs: Chlorine:	ABC reagent water produced using reverse-osmosis water passed through a series of deionization tanks, a laboratory water purification system consisting of carbon, de-mineralization, and organic adsorption cartridges, and then through a 0.2-µm filter. Not reported Not reported Not reported Not reported N/A RO water Not reported Not reported Not reported Some detected (see remarks) None detected at toxic levels Not detected (<0.05 mg/L; June 2015)	Water analyses were performed on samples collected on June 2015. <u>Metals (mg/L)</u> Barium (0.0196) Boron (0.377) Calcium (60.2) Iron (1.01) Magnesium (26.5) Manganese (0.0150) Potassium (7.37) Sodium (27.5) Zinc (0.117) Lead (0.0017) *Water used for preparation of nutrient medium should be of reagent quality (e.g., ASTM Type I water).* *Marine algal nutrient medium is prepared by adding reagent grade chemicals to synthetic salt water or filtered natural salt water, or by preparing a complete saltwater medium. Salinity for saltwater medium should be 30 ± 5 ‰.*
Indicate how the test material is added to the medium (added directly or used stock solution)	A 0.03 mg/mL primary stock solution was prepared by bringing 68.7 mg (30 mg corrected for purity) of the test substance to 1L with freshwater algal medium with sodium silicate (FWAM+Si). The remaining solutions were prepared by further diluting	All test concentrations were calculated as the 2,4-DB DMAS acid and were designated as 2,4-DB DMAS acid equivalents (mg 2,4-DB DMAS ae/L). For the control, test medium was used without addition of the test item.

Parameter	**Details**	**Remarks**
		Criteria
	the test solution with 1.0 L of test medium.	
Aeration or agitation Oscillation rate:	Continuous swirling on orbital shaker table 100 rpm	 *EPA recommends rotary shaking apparatus to oscillate vessels at approximately 100 cycles/min during the test. The rate of oscillation should be determined at test initiation or at least once daily during testing if the shaking rate changes. S. costatum should be shaken by hand 1-2X daily or shaken at 60 cycles/min.*
Initial cell density	1.0 x 10^4 algal cells/mL	None *EPA recommends an initial population density of 10,000 cells/mL for P. subcapitata, S. costatum and at a minimum 10,000 cells/mL for all other test species. Other species may need a higher initial inoculum density and should be determined on a case-by case basis.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates Negative control: Solvent control: Treatments:	 4 N/A 4	An additional abiotic 0.94 mg ae/L replicate was prepared without algae and used to evaluate the potential for incorporation of the test substance into the algal biomass. *EPA recommends a minimum number of 4 replicates per treatment and control/ solvent control.*

Parameter	**Details**	**Remarks**
		Criteria
		OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test. *EPA recommends treatments be randomly assigned to test vessels, and test vessels randomly assigned to positions in the growth chamber.*
<u>Test concentrations</u> Nominal: Measured:	0 (negative control), 0.94, 1.9, 3.8, 7.5, 15, and 30 mg ae/L <0.04 (<MQL, control), 1.03, 2.12, 4.21, 8.47, 17.0, and 32.8 mg ae/L	None
		EPA recommends at least 5 test concentrations, in geometric series with a ratio of 2 to 4, and insure bracketing the NOAEC or IC_{05} and the IC_{50}, plus a control/solvent control. *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used):	N/A	*EPA recommends the solvent N,N-dimethyl-formamide. The concentration of solvent should be the same in all test treatments and should not exceed 0.1 mL/L.*
Method and interval of analytical verification	The 0-hour samples were collected from parent solutions, and the 96-hour biotic samples were collected after compositing replicate solutions from each treatment and control	The method LOQ was 0.0408 mg ae/L. 96-h recoveries from QC samples fortified at 0.450 and 36.3 mg ae/L were 102 and 120%, respectively.

Parameter	Details	Remarks
		Criteria
	group. Sample analysis was performed by LC-MS/MS.	*EPA recommends confirmation of dissolved test concentrations at a minimum at test initiation and at test termination for static tests.*
<u>Test conditions</u> pH: Temperature (media): Temperature (air): Photoperiod: Light intensity and quality:	7.4 to 7.5 at initiation 23.5 to 24.6ºC 24 ± 2°C Continuous Cool-white fluorescent lighting; 4724 to 4736 lux	*<u>EPA Recommendations</u>* *pH at test initiation: 7.5±0.1 for freshwater and 8.0±0.1 for marine. Temperature for P. subcapita and N. pelliculosa is 24±2 °C, and for S. costatum is 20±2 ºC. Photoperiod for P. subcapita and N. pelliculosa is continuous, and for S. costatum is 14 hr light/ 10 hr dark. Light intensity: 60 µmol/m2/s or 4300 lux.* *OECD recommended the temperature in the range of 21 to 25ºC maintained at ± 2ºC and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector. OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.* *EPA recommends measuring pH at test initiation and at end of the test (or daily if pH adjustment was necessary); temperature on a separate vessel or hourly/daily on the air; and light intensity at test initiation (or daily if intensity changed by >15%);*
<u>Reference chemical (if used)</u> Name: Concentrations:	 N/A N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observation parameters

Parameters	**Details**	**Remarks** **Criteria**
Parameters measured including growth inhibition/other toxicity symptoms:	Algal cell density, yield, growth rate, and area under the growth curve (AUC)	None *Recommended parameters measured per replicate include:* *-Algal cell density (cell count/mL)* *-yield (final population density)* *-average specific growth rate* *-mean area under growth curve (AUC)*
Measurement technique for cell density and other end points	Cell density was measured by direct microscopic counting with a hemacytometer. Growth rate was calculated from cell density using a logarithmic growth equation. Yield was calculated as final minus initial cell density. Area under the curve was calculated as the area between the growth curves.	None *EPA recommends the measurement of cell counts by microscopic observation or electronic particle counter, with alternative option of measuring chlorophyll a.* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Cell densities were determined every 24 hours.	None *EPA and OECD: every 24 hours.*
Other observations, if any	N/A	N/A

Parameters	Details	Remarks Criteria
Indicate whether there was an exponential growth in the control	Yes, mean cell density in the control group increased by a factor of 139X after 96 hours.	None *During the 96 hour test period, cell counts in the controls did not increase by a factor of at least 100X for P. subcapitata and a factor of at least 30X for S. costatum (i.e., logarithmic growth in the controls was not reached during the test).* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	N/A

II. RESULTS and DISCUSSION:

A. STUDY AUTHORS RESULTS:

After 96 hours, the mean cell density of the negative control was 139 x 10^4 cells/mL, yielding inhibitions relative to the negative control of -2, -2, -1, 38, 92, and 96% for mean measured concentrations of 1.03, 2.12, 4.21, 8.47, 17.0, and 32.8 mg ae/L, respectively (see **Table 3**). The study author did not calculate or analyze cell density values.

After 96 hours, area under the growth curve (AUC) was inhibited -1, -3, 0, 44, 85, and 93% relative to the negative control at the mean-measured 1.03, 2.12, 4.21, 8.47, 17.0, and 32.8 mg ae/L treatment levels, respectively (see **Table 4**). Inhibition of growth rate after 96 hours was 0, 0, 0, 10, 52, and 64% relative to the negative control for the mean-measured 1.03, 2.12, 4.21, 8.47, 17.0, and 32.8 mg ae/L treatment levels, respectively. Yield after 96 hours was inhibited -2, -2, -1, 38, 93, and 96% relative to the negative control at the mean-measured 1.03, 2.12, 4.21, 8.47, 17.0, and 32.8 mg ae/L treatment levels, respectively.

For a satisfactory test, cell counts in the controls should increase by a factor of at least 100X for P. subcapitata and a factor of at least 30X for S. costatum by test termination (i.e., logarithmic growth in the controls). At test termination the coefficient of variation (CV) for mean control yield should be < 15% and the CV for average specific growth rate should be < 15%, which is a logarithmically-transformed variable.

Table 3: Study Author Reported Effects of 2,4-DB DMAS on Freshwater Diatom (*Navicula pelliculosa*) Cell Density

Nominal/Mean-Measured/ Concentrations (mg ae/L)	Initial cell density (cells/mL x 10^4)	Cell Density (cells/mL x 10^4)			% Inhibition
		48 h	72 h	96 h	
Negative control	1.0	12.0	46.9	139	N/A
0.94/1.03	1.0	13.0	46.3	141	-2
1.9/2.12	1.0	13.2	48.5	141	-2

3.8/4.21	1.0	12.9	45.6	139	-1
7.5/8.47	1.0	7.72*	22.5*	85.7*	38
15/17.0	1.0	6.00*	9.41*	10.6*	92
30/32.8	1.0	4.00*	3.95*	6.00*	96

* Significantly reduced compared to the negative control (Dunnett's test, p= 0.05).

Table 4: Study Author-Reported Effects of 2,4-DB DMAS on Freshwater Diatom (*Navicula pelliculosa*) Growth

Nominal/Mean-Measured Concentrations (mg ae/L)	Mean Growth Rate (day^{-1})		Mean AUC		Mean Yield ($x10^4$ cells/mL)	
	0-96 hours	% Inhibition	0-96 hours	% Inhibition	0-96 hours	% Inhibition
Negative control	0.052	N/A	3,060	N/A	138	N/A
0.94/1.03	0.052	0	3,100	-1	140	-2
1.9/2.12	0.052	0	3,160	-3	140	-2
3.8/4.21	0.052	0	3,060	0	138	-1
7.5/8.47	0.047*	10	1,730*	44	84.7*	38
15/17.0	0.025*	52	458*	85	9.57*	93
30/32.8	0.019*	64	207*	93	5.01*	96

* Significantly reduced compared to the negative control (Dunnett's test, p= 0.05).

B. REPORTED STATISTICS:

The study author's statistical analyses were performed on 72- and 96-hour area under the growth curve (AUC), growth rate, and yield data using SAS (Version 9.3) statistical software.

All endpoints were evaluated for normality and homogeneity of variance (p=0.01) using Shapiro-Wilk's and Levene's tests, respectively. The NOAEC values, which were based on area under the growth curve (AUC), growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test (p=0.05) where the alternate hypothesis was that the mean for the growth parameter was reduced in comparison to the control. Non-parametric analyses were performed on area cell density, growth rate, and yield data at 48 hours. Parametric analyses were performed on cell density, growth rate, and yield data at 24, 72, and 96-hour data, as well as for all area under the growth curve data. Inhibitory concentrations (IC_x) and their corresponding 95% confidence intervals were estimated using non-linear regression models.

Note that the study author used nominal concentrations for all reporting and analyses, including for estimation of the following endpoints:

Yield

IC_{10}: 5.04 mg ae/L 95% C.I.: 4.79-5.28 mg ae/L
IC_{20}: 6.07 mg ae/L 95% C.I.: 5.86-6.29 mg ae/L
IC_{50}: 8.35 mg ae/L 95% C.I.: 8.17-8.54 mg ae/L
NOAEC: 3.8 mg ae/L LOAEC: 7.5 mg ae/L

Growth Rate

IC_{10}: 4.14 mg ae/L 95% C.I.: 2.6-5.68 mg ae/L
IC_{20}: 7.18 mg ae/L 95% C.I.: 5.35-9.01 mg ae/L
IC_{50}: 18.4 mg ae/L 95% C.I.: 16.4-20.5 mg ae/L
NOAEC: 3.8 mg ae/L LOAEC: 7.5 mg ae/L

Area Under the Growth Rate Curve (AUC)

IC_{10}: 3.8 mg ae/L	95% C.I.: 3.35-4.25 mg ae/L
IC_{20}: 5.08 mg ae/L	95% C.I.: 4.63-5.52 mg ae/L
IC_{50}: 8.31 mg ae/L	95% C.I.: 7.91-8.71 mg ae/L
NOAEC: 3.8 mg ae/L	LOAEC: 7.5 mg ae/L

C. VERIFICATION OF STATISTICAL RESULTS:

Reviewers verified that the cell density data in **Table 3** accurately matches the results reported by the study author. The parameters (*i.e.*, growth rate, yield, and AUC) calculated by the study author and given in **Table 4** were compared with calculations for these same parameters made by the reviewer based on the raw cell density data. While mean yield results calculated by the reviewer matched that reported by the study author (see **Table 4**), results for growth rate and AUC calculated by the reviewer (see **Table 5**) differed from those of the study author.

Table 5: Reviewer-Calculated Effects of 2,4-DB DMAS on Freshwater Diatom (*Navicula pelliculosa*) Growth

Nominal/Mean-Measured Concentrations (mg ae/L)	Mean Growth Rate (day^{-1})		Mean AUC ($x10^4$ cells x days/mL)		Mean Yield ($x10^4$ cells/mL)	
	0-96 hours	% Inhibition	0-96 hours	% Inhibition	0-96 hours	% Inhibition
Negative control	1.23	N/A	127.66	N/A	138	N/A
0.94/1.03	1.24	0	129.25	-1	140	-2
1.9/2.12	1.24	0	131.76	-3	140	-2
3.8/4.21	1.23	0	127.66	0	138	-1
7.5/8.47	1.11†	10	72.03*	44	84.7*	38
15/17.0	0.59†	52	19.05*	85	9.56*	93
30/32.8	0.45†	64	8.61*	93	5.00*	96

* Significantly reduced compared to the negative control (Dunnett's test, p= 0.05).

† Significantly reduced compared to the negative control (Mann-Whitney U Two Sample test, p= 0.05).

The toxicity effects of formulated 2,4-DB on *N. pelliculosa* were analyzed using CETIS™ statistical software (version 1.9.2.8 with database backend settings implemented by EFED on October 20, 2015) (see **Appendix I** for CETIS™ reports).

Data were initially assessed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. Yield and area under the curve data met both assumptions and were therefore analyzed using ANOVA followed by Dunnett's test. Growth rate data did not meet assumptions for parametric tests, and so were analyzed using the Mann-Whitney U Two-Sample test. IC_x values were estimated using the standard linear log-normal (Probit) regression model. All analyses were conducted using mean-measured test concentrations.

Yield

IC_{05}: 4.96 mg ae/L	95% C.I.: 4.62-5.24 mg ae/L
IC_{50}: 9.44 mg ae/L	95% C.I.: 9.24-9.64 mg ae/L
NOAEC: 4.21 mg ae/L	LOAEC: 7.5 mg ae/L

Growth rate

IC_{05}: 4.47 mg ae/L	95% C.I.: 3.08-5.56 mg ae/L

IC_{50}: 19.99 mg ae/L 95% C.I.: 18.26-21.88 mg ae/L
NOAEC: 4.21 mg ae/L LOAEC: 7.5 mg ae/L

Area under the curve (AUC)
IC_{05}: 3.9 mg ae/L* 95% C.I.: 3.34-4.32 mg ae/L
IC_{50}: 9.38 mg ae/L 95% C.I.: 8.97-9.8 mg ae/L
NOAEC: 4.21 mg ae/L LOAEC: 7.5 mg ae/L

Note that the IC_{05} value for area under the growth curve is below the estimated NOAEC value, but that the NOAEC falls within the 95% CI for the estimated IC_{05} value. This endpoint should only be used with caution.

Endpoint(s) Affected: Yield, growth rate, and area under the curve
Most Sensitive Endpoint: Area under the curve

D. REVIEWER'S COMMENTS:
The experimental phase of the definitive test was conducted from 19 to 23 October 2015. All core guideline validity requirements (OCSPP 850.4500) appear to have been met by this study. Specifically, the coefficients of variation (CV) of the negative control for the yield and growth rate parameters were 0.42 and 0.0%, respectively, which meets the guideline requirements of CV<15%.

The reviewers' toxicity endpoints were similar to those reported by the study author when taking into account that the study author's toxicity values were reported in terms of nominal concentrations whereas the reviewers' toxicity values were reported in terms of mean-measured concentrations. Also, while the reviewers' and study authors' calculations of cell density yield and area under the growth rate curve (AUC) resulted in different values, statistical analyses arrived at the same toxicity endpoints for these parameters. The reviewer's results are presented in the Executive Summary and Conclusions sections of this report.

E. CONCLUSIONS:
This study **is scientifically sound** and is classified as **acceptable**. At 96 hours, the most sensitive endpoint was area under the growth curve (AUC), with an IC_{50} (with 95% C.I.) of 9.38 (8.97 to 9.80) mg ae/L, and a NOAEC and LOAEC, respectively, of 4.21 and 7.5 mg ae/L.

III. REFERENCES:

American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM E1218-97a. 14 pp.

Schwenke, J. and Milliken, G. 1991. On the Calibration Problem Extended to Nonlinear Models. Biometrics, Vol. 47, No. 2: 563-574.

CETIS Summary Report

OCSPP 850.4500 Algal Toxicity — **ABC Labs**

Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD	✓
08-9538-6078	96h AUC	Dunnett Multiple Comparison Test	4.21	8.47	5.971		3.35%	✓
00-1841-7071	96h AUC	Williams Multiple Comparison Test	4.21	8.47	5.971		2.54%	✓
10-3046-3375	96h Cell Density	Dunnett Multiple Comparison Test	4.21	8.47	5.971		2.52%	✓
18-5201-3814	96h Cell Density	Williams Multiple Comparison Test	4.21	8.47	5.971		1.91%	✓
05-8542-2197	96h Growth Rate	Jonckheere-Terpstra Step-Down Test	4.21	8.47	5.971		n/a	✓
03-2675-2351	96h Growth Rate	Mann-Whitney U Two-Sample Test	4.21	8.47	5.971		1.25%	✓

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	mg ae/L	95% LCL	95% UCL	TU	✓
15-9024-2586	96h AUC	Regression: 3P Cum Log-Normal (Probit)	IC5	3.899	3.343	4.324		✓
			IC10	4.733	4.267	5.141		✓
			IC25	6.542	6.14	6.938		✓
			IC50	9.375	8.972	9.795		✓
13-5231-1319	96h Cell Density	Regression: 3P Cum Log-Normal (Probit)	IC5	4.96	4.624	5.235		
			IC10	5.717	5.439	5.967		
			IC25	7.248	7.041	7.451		
			IC50	9.436	9.24	9.635		
09-7344-9992	96h Growth Rate	Regression: 3P Cum Log-Normal (Probit)	IC5	4.469	3.078	5.56		
			IC10	6.222	4.938	7.401		
			IC25	10.82	9.484	12.19		
			IC50	19.99	18.26	21.88		

OCSPP 850.4500 Algal Toxicity **ABC Labs**

96h AUC Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	127.7	125	130.4	126	130	0.8498	1.7	1.33%	0.00%
1.03		4	129.2	123.5	135	126	133.9	1.811	3.622	2.80%	-1.25%
2.12		4	131.8	129	134.5	129.8	133.2	0.8524	1.705	1.29%	-3.21%
4.21		4	127.7	123.7	131.6	125.1	130.1	1.251	2.502	1.96%	0.00%
8.47		4	72.02	67.75	76.3	68.14	74.3	1.344	2.688	3.73%	43.58%
17		4	19.05	14.13	23.97	16.01	22.82	1.546	3.092	16.23%	85.08%
32.8		4	8.61	7.178	10.04	7.61	9.77	0.4501	0.9001	10.45%	93.26%

96h Cell Density Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	137.5	136.6	138.4	137	138	0.2887	0.5774	0.42%	0.00%
1.03		4	140	134	146	135	144	1.871	3.742	2.67%	-1.82%
2.12		4	140	136.3	143.7	138	142	1.155	2.309	1.65%	-1.82%
4.21		4	138.2	135.2	141.3	137	141	0.9465	1.893	1.37%	-0.55%
8.47		4	84.65	81.56	87.74	82.5	87	0.97	1.94	2.29%	38.44%
17		4	9.555	8.086	11.02	8.22	10.3	0.4615	0.9231	9.66%	93.05%
32.8		4	5.002	4.28	5.725	4.67	5.67	0.227	0.454	9.07%	96.36%

96h Growth Rate Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	1.23	1.23	1.23	1.23	1.23	0	0	0.00%	0.00%
1.03		4	1.238	1.23	1.245	1.23	1.24	0.0025	0.005	0.40%	-0.61%
2.12		4	1.235	1.226	1.244	1.23	1.24	0.002887	0.005774	0.47%	-0.41%
4.21		4	1.232	1.225	1.24	1.23	1.24	0.0025	0.005	0.41%	-0.20%
8.47		4	1.113	1.105	1.12	1.11	1.12	0.0025	0.005	0.45%	9.55%
17		4	0.59	0.5556	0.6244	0.56	0.61	0.0108	0.0216	3.66%	52.03%
32.8		4	0.445	0.4174	0.4726	0.43	0.47	0.00866	0.01732	3.89%	63.82%

OCSPP 850.4500 Algal Toxicity **ABC Labs**

96h AUC Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	127.4	126	130	127.2
1.03		130.3	126	133.9	126.8
2.12		133.2	133.1	130.9	129.8
4.21		129.4	126	130.1	125.1
8.47		74.3	72.59	73.07	68.14
17		20.26	16.01	22.82	17.11
32.8		8.72	7.61	8.34	9.77

96h Cell Density Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	137	138	137	138
1.03		140	135	144	141
2.12		138	138	142	142
4.21		138	137	141	137
8.47		87	85.3	83.8	82.5
17		10.3	8.22	9.7	10
32.8		4.78	5.67	4.67	4.89

96h Growth Rate Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.23	1.23	1.23	1.23
1.03		1.24	1.23	1.24	1.24
2.12		1.23	1.23	1.24	1.24
4.21		1.23	1.23	1.24	1.23
8.47		1.12	1.11	1.11	1.11
17		0.61	0.56	0.59	0.6
32.8		0.44	0.47	0.43	0.44

OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	00-1841-7071	**Endpoint:**	96h AUC	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Parametric-Control vs Ord.Treatments	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	4.21	8.47	5.971		2.54%

Williams Multiple Comparison Test

Control vs	Conc-mg ae/	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	1.03	-0.9097	1.721	3.008	6	CDF	>0.05	Non-Significant Effect
	2.12	-1.628	1.802	3.15	6	CDF	>0.05	Non-Significant Effect
	4.21	0	1.829	3.197	6	CDF	>0.05	Non-Significant Effect
	8.47*	31.83	1.842	3.22	6	CDF	<0.05	Significant Effect
	17*	62.13	1.85	3.234	6	CDF	<0.05	Significant Effect
	32.8*	68.11	1.855	3.243	6	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	72291.9	12048.7	6	1972	<1.0E-37	Significant Effect
Error	128.32	6.11048	21			
Total	72420.2		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	5.614	16.81	0.4678	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9791	0.8975	0.8287	Normal Distribution

96h AUC Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	127.7	125	130.4	127.3	126	130	0.8499	1.33%	0.00%
1.03		4	129.2	123.5	135	128.5	126	133.9	1.811	2.80%	-1.25%
2.12		4	131.8	129	134.5	132	129.8	133.2	0.8524	1.29%	-3.21%
4.21		4	127.7	123.7	131.6	127.7	125.1	130.1	1.251	1.96%	0.00%
8.47		4	72.02	67.75	76.3	72.83	68.14	74.3	1.344	3.73%	43.58%
17		4	19.05	14.13	23.97	18.69	16.01	22.82	1.546	16.23%	85.08%
32.8		4	8.61	7.178	10.04	8.53	7.61	9.77	0.4501	10.45%	93.26%

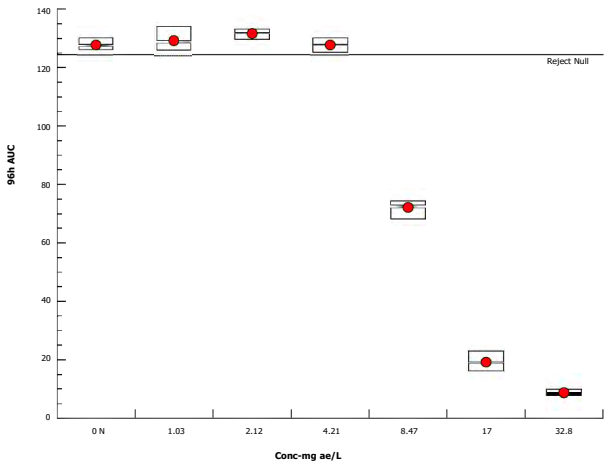
OCSPP 850.4500 Algal Toxicity **ABC Labs**

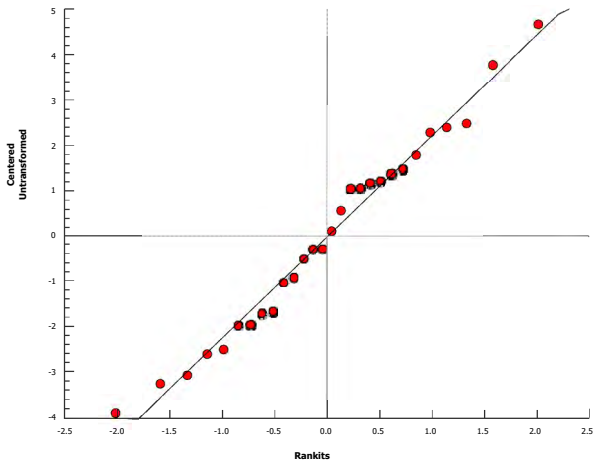
Analysis ID: 00-1841-7071 **Endpoint:** 96h AUC **CETIS Version:** CETISv1.9.2
Analyzed: 20 Jul-17 16:02 **Analysis:** Parametric-Control vs Ord.Treatments **Official Results:** Yes

96h AUC Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	127.4	126	130	127.2
1.03		130.3	126	133.9	126.8
2.12		133.2	133.1	130.9	129.8
4.21		129.4	126	130.1	125.1
8.47		74.3	72.59	73.07	68.14
17		20.26	16.01	22.82	17.11
32.8		8.72	7.61	8.34	9.77

Graphics





OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	08-9538-6078	**Endpoint:**	96h AUC	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:03	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	4.21	8.47	5.971		3.35%

Dunnett Multiple Comparison Test

Control vs	Conc-mg ae/	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	1.03	-0.9097	2.448	4.279	6	CDF	0.9835	Non-Significant Effect
	2.12	-2.347	2.448	4.279	6	CDF	0.9998	Non-Significant Effect
	4.21	0	2.448	4.279	6	CDF	0.8571	Non-Significant Effect
	8.47*	31.83	2.448	4.279	6	CDF	6.3E-07	Significant Effect
	17*	62.13	2.448	4.279	6	CDF	6.3E-07	Significant Effect
	32.8*	68.11	2.448	4.279	6	CDF	6.3E-07	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	72291.9	12048.7	6	1972	<1.0E-37	Significant Effect
Error	128.32	6.11048	21			
Total	72420.2		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	5.614	16.81	0.4678	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9791	0.8975	0.8287	Normal Distribution

96h AUC Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	127.7	125	130.4	127.3	126	130	0.8499	1.33%	0.00%
1.03		4	129.2	123.5	135	128.5	126	133.9	1.811	2.80%	-1.25%
2.12		4	131.8	129	134.5	132	129.8	133.2	0.8524	1.29%	-3.21%
4.21		4	127.7	123.7	131.6	127.7	125.1	130.1	1.251	1.96%	0.00%
8.47		4	72.02	67.75	76.3	72.83	68.14	74.3	1.344	3.73%	43.58%
17		4	19.05	14.13	23.97	18.69	16.01	22.82	1.546	16.23%	85.08%
32.8		4	8.61	7.178	10.04	8.53	7.61	9.77	0.4501	10.45%	93.26%

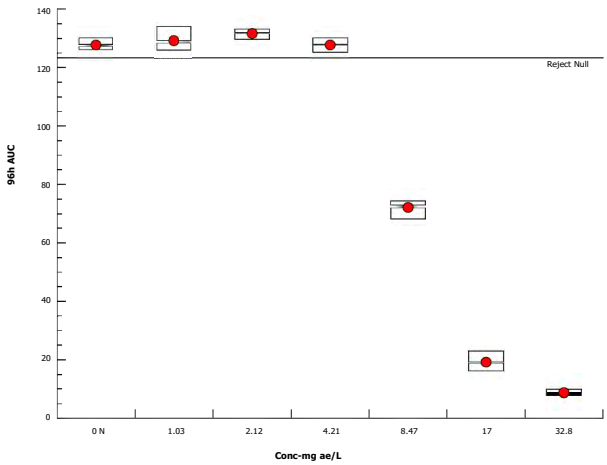
OCSPP 850.4500 Algal Toxicity **ABC Labs**

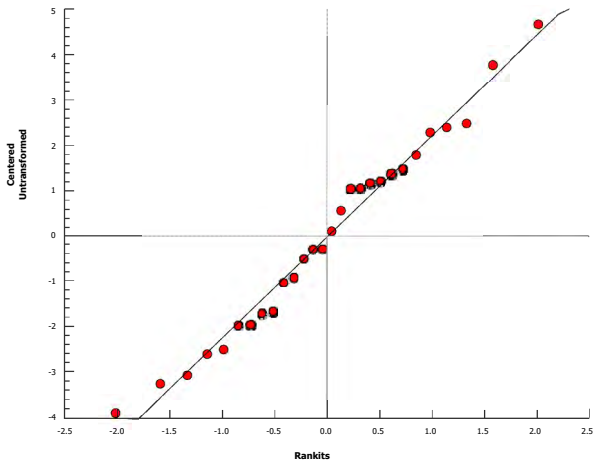
Analysis ID: 08-9538-6078 **Endpoint:** 96h AUC **CETIS Version:** CETISv1.9.2
Analyzed: 20 Jul-17 16:03 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

96h AUC Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	127.4	126	130	127.2
1.03		130.3	126	133.9	126.8
2.12		133.2	133.1	130.9	129.8
4.21		129.4	126	130.1	125.1
8.47		74.3	72.59	73.07	68.14
17		20.26	16.01	22.82	17.11
32.8		8.72	7.61	8.34	9.77

Graphics





OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	18-5201-3814	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Parametric-Control vs Ord.Treatments	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	4.21	8.47	5.971		1.91%

Williams Multiple Comparison Test

Control	vs	Conc-mg ae/	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.03	-1.766	1.721	2.437	6	CDF	>0.05	Non-Significant Effect
		2.12	-1.766	1.802	2.552	6	CDF	>0.05	Non-Significant Effect
		4.21	-0.5297	1.829	2.59	6	CDF	>0.05	Non-Significant Effect
		8.47*	37.32	1.842	2.609	6	CDF	<0.05	Significant Effect
		17*	90.36	1.85	2.62	6	CDF	<0.05	Significant Effect
		32.8*	93.57	1.855	2.627	6	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	92879.7	15480	6	3860	<1.0E-37	Significant Effect
Error	84.2146	4.01022	21			
Total	92963.9		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	15.03	16.81	0.0200	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9635	0.8975	0.4206	Normal Distribution

96h Cell Density Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	137.5	136.6	138.4	137.5	137	138	0.2887	0.42%	0.00%
1.03		4	140	134	146	140.5	135	144	1.871	2.67%	-1.82%
2.12		4	140	136.3	143.7	140	138	142	1.155	1.65%	-1.82%
4.21		4	138.2	135.2	141.3	137.5	137	141	0.9465	1.37%	-0.55%
8.47		4	84.65	81.56	87.74	84.55	82.5	87	0.97	2.29%	38.44%
17		4	9.555	8.086	11.02	9.85	8.22	10.3	0.4615	9.66%	93.05%
32.8		4	5.003	4.28	5.725	4.835	4.67	5.67	0.227	9.07%	96.36%

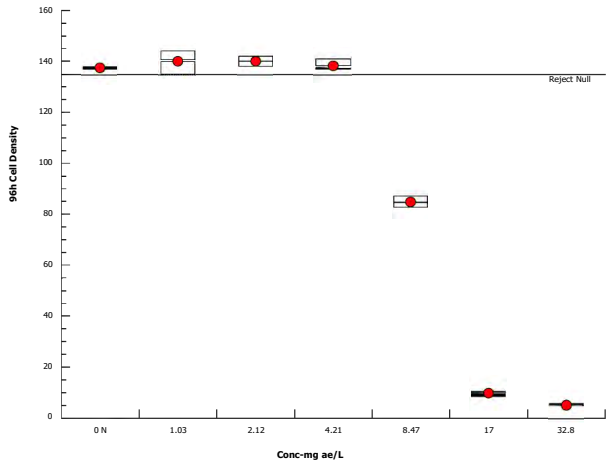
OCSPP 850.4500 Algal Toxicity **ABC Labs**

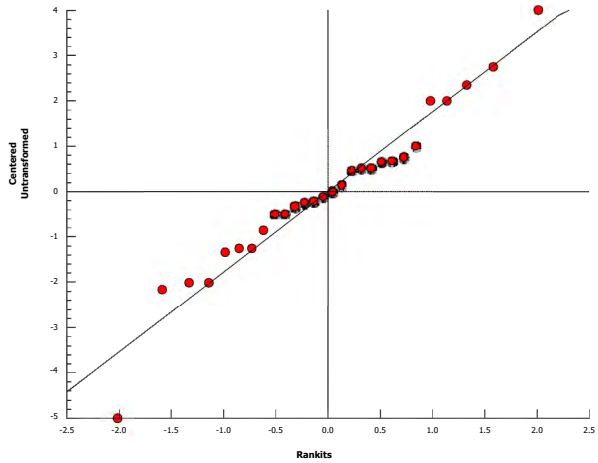
Analysis ID:	18-5201-3814	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Parametric-Control vs Ord.Treatments	**Official Results:**	Yes

96h Cell Density Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	137	138	137	138
1.03		140	135	144	141
2.12		138	138	142	142
4.21		138	137	141	137
8.47		87	85.3	83.8	82.5
17		10.3	8.22	9.7	10
32.8		4.78	5.67	4.67	4.89

Graphics





OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	10-3046-3375	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:03	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	4.21	8.47	5.971		2.52%

Dunnett Multiple Comparison Test

Control	vs	Conc-mg ae/	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.03	-1.766	2.448	3.466	6	CDF	0.9988	Non-Significant Effect
		2.12	-1.766	2.448	3.466	6	CDF	0.9988	Non-Significant Effect
		4.21	-0.5297	2.448	3.466	6	CDF	0.9550	Non-Significant Effect
		8.47*	37.32	2.448	3.466	6	CDF	6.3E-07	Significant Effect
		17*	90.36	2.448	3.466	6	CDF	6.3E-07	Significant Effect
		32.8*	93.57	2.448	3.466	6	CDF	6.3E-07	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	92879.7	15480	6	3860	<1.0E-37	Significant Effect
Error	84.2146	4.01022	21			
Total	92963.9		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	15.03	16.81	0.0200	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9635	0.8975	0.4206	Normal Distribution

96h Cell Density Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	137.5	136.6	138.4	137.5	137	138	0.2887	0.42%	0.00%
1.03		4	140	134	146	140.5	135	144	1.871	2.67%	-1.82%
2.12		4	140	136.3	143.7	140	138	142	1.155	1.65%	-1.82%
4.21		4	138.2	135.2	141.3	137.5	137	141	0.9465	1.37%	-0.55%
8.47		4	84.65	81.56	87.74	84.55	82.5	87	0.97	2.29%	38.44%
17		4	9.555	8.086	11.02	9.85	8.22	10.3	0.4615	9.66%	93.05%
32.8		4	5.003	4.28	5.725	4.835	4.67	5.67	0.227	9.07%	96.36%

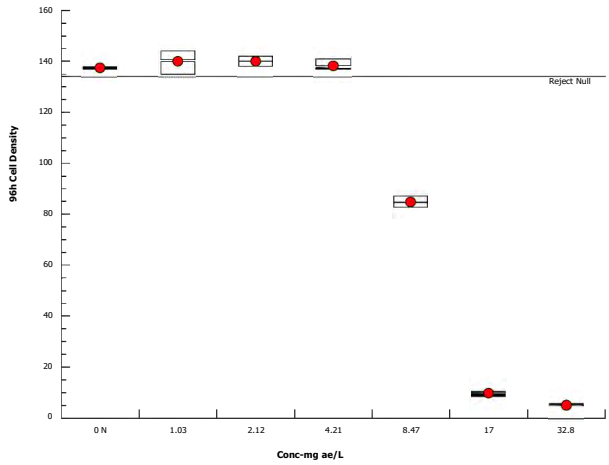
OCSPP 850.4500 Algal Toxicity **ABC Labs**

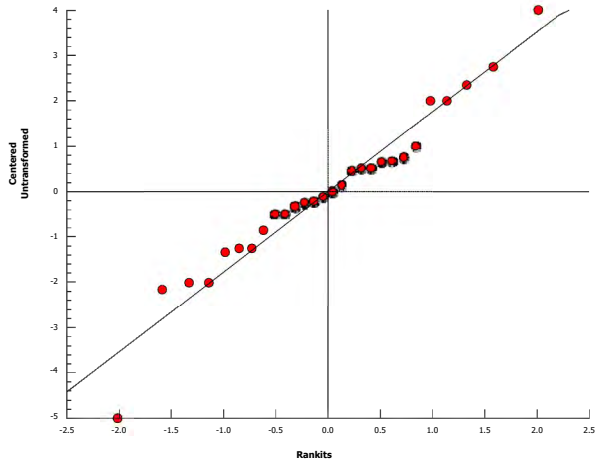
Analysis ID:	10-3046-3375	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:03	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

96h Cell Density Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	137	138	137	138
1.03		140	135	144	141
2.12		138	138	142	142
4.21		138	137	141	137
8.47		87	85.3	83.8	82.5
17		10.3	8.22	9.7	10
32.8		4.78	5.67	4.67	4.89

Graphics





OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	05-8542-2197	**Endpoint:**	96h Growth Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	4.21	8.47	5.971	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-mg ae/	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	1.03	-2.049	1.645	2	Asymp	0.9798	Non-Significant Effect
	2.12	-1.373	1.645	2	Asymp	0.9152	Non-Significant Effect
	4.21	-0.4472	1.645	2	Asymp	0.6726	Non-Significant Effect
	8.47*	2.03	1.645	3	Asymp	0.0212	Significant Effect
	17*	3.602	1.645	3	Asymp	1.6E-04	Significant Effect
	32.8*	4.785	1.645	4	Asymp	8.6E-07	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.82559	0.470931	6	3767	<1.0E-37	Significant Effect
Error	0.002625	0.000125	21			
Total	2.82821		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	2.947	3.812	0.0302	Equal Variances
Variances	Mod Levene Equality of Variance Test	1.649	3.812	0.1832	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8894	0.8975	0.0065	Non-Normal Distribution

96h Growth Rate Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.23	1.23	1.23	1.23	1.23	1.23	0	0.00%	0.00%
1.03		4	1.238	1.23	1.245	1.24	1.23	1.24	0.0025	0.40%	-0.61%
2.12		4	1.235	1.226	1.244	1.235	1.23	1.24	0.002886	0.47%	-0.41%
4.21		4	1.233	1.225	1.24	1.23	1.23	1.24	0.002498	0.41%	-0.20%
8.47		4	1.113	1.105	1.12	1.11	1.11	1.12	0.002499	0.45%	9.55%
17		4	0.59	0.5556	0.6244	0.595	0.56	0.61	0.0108	3.66%	52.03%
32.8		4	0.445	0.4174	0.4726	0.44	0.43	0.47	0.00866	3.89%	63.82%

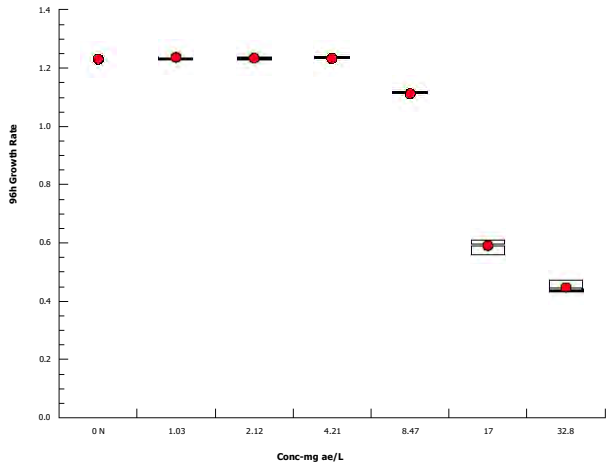
OCSPP 850.4500 Algal Toxicity **ABC Labs**

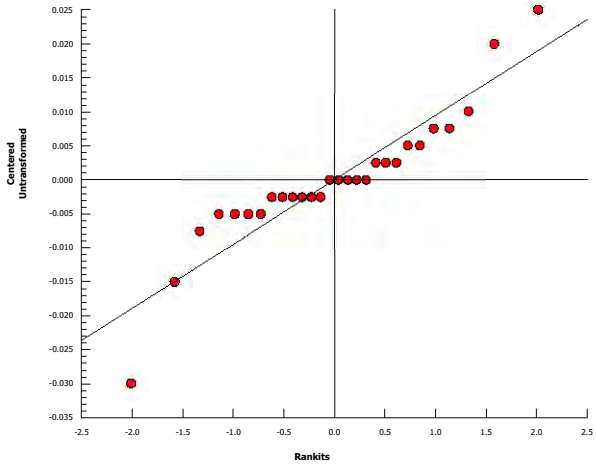
Analysis ID: 05-8542-2197 **Endpoint:** 96h Growth Rate **CETIS Version:** CETISv1.9.2
Analyzed: 20 Jul-17 16:02 **Analysis:** Nonparametric-Control vs Ord. Treatments **Official Results:** Yes

96h Growth Rate Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.23	1.23	1.23	1.23
1.03		1.24	1.23	1.24	1.24
2.12		1.23	1.23	1.24	1.24
4.21		1.23	1.23	1.24	1.23
8.47		1.12	1.11	1.11	1.11
17		0.61	0.56	0.59	0.6
32.8		0.44	0.47	0.43	0.44

Graphics





OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	03-2675-2351	**Endpoint:**	96h Growth Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:03	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	4.21	8.47	5.971		1.25%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-mg ae/	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.03	2	n/a	1	6	Exact	1.0000	Non-Significant Effect
		2.12	4	n/a	1	6	Exact	1.0000	Non-Significant Effect
		4.21	6	n/a	1	6	Exact	1.0000	Non-Significant Effect
		8.47*	16	n/a	0	6	Exact	0.0143	Significant Effect
		17*	16	n/a	0	6	Exact	0.0143	Significant Effect
		32.8*	16	n/a	0	6	Exact	0.0143	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.82559	0.470931	6	3767	<1.0E-37	Significant Effect
Error	0.002625	0.000125	21			
Total	2.82821		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	2.947	3.812	0.0302	Equal Variances
Variances	Mod Levene Equality of Variance Test	1.649	3.812	0.1832	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8894	0.8975	0.0065	Non-Normal Distribution

96h Growth Rate Summary

Conc-mg ae/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.23	1.23	1.23	1.23	1.23	1.23	0	0.00%	0.00%
1.03		4	1.238	1.23	1.245	1.24	1.23	1.24	0.0025	0.40%	-0.61%
2.12		4	1.235	1.226	1.244	1.235	1.23	1.24	0.002886	0.47%	-0.41%
4.21		4	1.233	1.225	1.24	1.23	1.23	1.24	0.002498	0.41%	-0.20%
8.47		4	1.113	1.105	1.12	1.11	1.11	1.12	0.002499	0.45%	9.55%
17		4	0.59	0.5556	0.6244	0.595	0.56	0.61	0.0108	3.66%	52.03%
32.8		4	0.445	0.4174	0.4726	0.44	0.43	0.47	0.00866	3.89%	63.82%

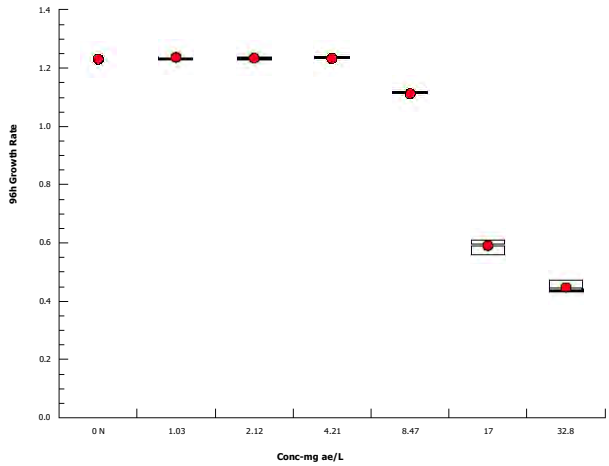
OCSPP 850.4500 Algal Toxicity **ABC Labs**

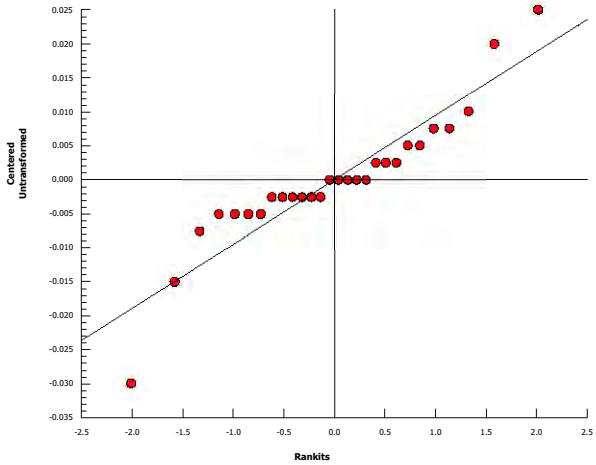
Analysis ID:	03-2675-2351	**Endpoint:**	96h Growth Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:03	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

96h Growth Rate Detail

Conc-mg ae/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.23	1.23	1.23	1.23
1.03		1.24	1.23	1.24	1.24
2.12		1.23	1.23	1.24	1.24
4.21		1.23	1.23	1.24	1.23
8.47		1.12	1.11	1.11	1.11
17		0.61	0.56	0.59	0.6
32.8		0.44	0.47	0.43	0.44

Graphics





OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	15-9024-2586	**Endpoint:**	96h AUC	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Nonlinear Regression (NLR)	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Cum Log-Normal (Probit): $\mu=\alpha\cdot[1-\Phi[\log[x/\delta]/\gamma]]$	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
9	-42.96	92.92	95.91	0.9913	Yes	18.51	2.84	0.0000	Significant Lack of Fit

Point Estimates

Level	mg ae/L	95% LCL	95% UCL
IC5	3.899	3.343	4.324
IC10	4.733	4.267	5.141
IC25	6.542	6.14	6.938
IC50	9.375	8.972	9.795

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	130.9	1.367	128	133.7	95.73	<1.0E-37	Significant Parameter
γ	0.5334	0.03244	0.4666	0.6002	16.44	<1.0E-37	Significant Parameter
δ	9.375	0.2179	8.926	9.824	43.02	<1.0E-37	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	288700	96220	3	4142	<1.0E-37	Significant
Lack of Fit	452.4	113.1	4	18.51	1.2E-06	Significant
Pure Error	128.3	6.11	21			
Residual	580.8	23.23	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance Test	5.614	12.59	0.4678	Equal Variances
	Mod Levene Equality of Variance	1.555	2.573	0.2096	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.953	0.9264	0.2351	Normal Distribution
	Anderson-Darling A2 Normality Te	0.4992	2.492	0.2135	Normal Distribution

OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID: 15-9024-2586 **Endpoint:** 96h AUC **CETIS Version:** CETISv1.9.2
Analyzed: 20 Jul-17 16:02 **Analysis:** Nonlinear Regression (NLR) **Official Results:** Yes

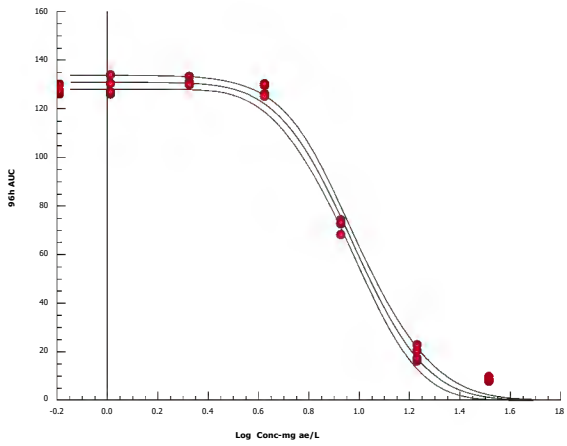
96h AUC Summary

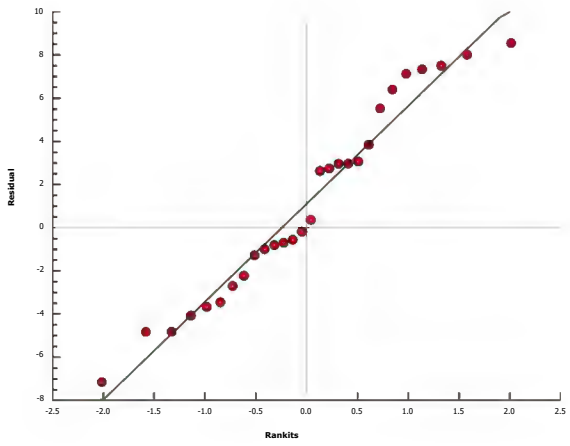
			Calculated Variate						
Conc-mg ae/L	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	127.7	126	130	0.8499	1.7	1.33%	0.0%
1.03		4	129.2	126	133.9	1.811	3.622	2.80%	-1.25%
2.12		4	131.8	129.8	133.2	0.8524	1.705	1.29%	-3.21%
4.21		4	127.7	125.1	130.1	1.251	2.502	1.96%	0.0%
8.47		4	72.02	68.14	74.3	1.344	2.688	3.73%	43.58%
17		4	19.05	16.01	22.82	1.546	3.092	16.23%	85.08%
32.8		4	8.61	7.61	9.77	0.4501	0.9001	10.45%	93.26%

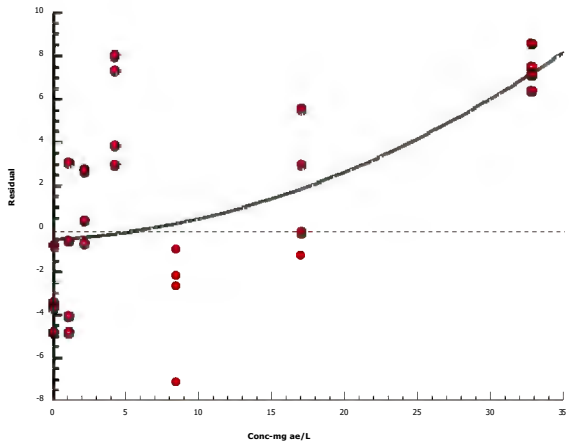
96h AUC Detail

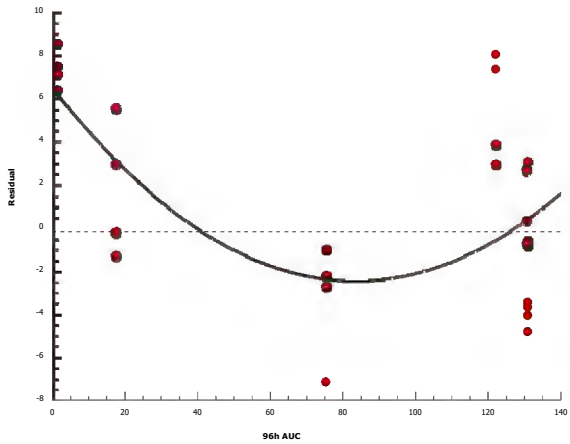
Conc-mg ae/L	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	127.4	126	130	127.2
1.03		130.3	126	133.9	126.8
2.12		133.2	133.1	130.9	129.8
4.21		129.4	126	130.1	125.1
8.47		74.3	72.59	73.07	68.14
17		20.26	16.01	22.82	17.11
32.8		8.72	7.61	8.34	9.77

Graphics Model: 3P Cum Log-Normal (Probit): μ=α·[1- Φ[log[x/δ]/γ]] Distribution: Normal [ω=1]









OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	13-5231-1319	**Endpoint:**	96h Cell Density	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Nonlinear Regression (NLR)	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Cum Log-Normal (Probit): μ=α·[1- Φ[log[x/δ]/γ]]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
6	-28.56	64.13	67.12	0.9976	Yes	7.698	2.84	0.0006	Significant Lack of Fit

Point Estimates

Level	mg ae/L	95% LCL	95% UCL
IC5	4.96	4.624	5.235
IC10	5.717	5.439	5.967
IC25	7.248	7.041	7.451
IC50	9.436	9.24	9.635

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	139.6	0.7591	138	141.1	183.9	<1.0E-37	Significant Parameter
γ	0.391	0.01764	0.3546	0.4273	22.17	<1.0E-37	Significant Parameter
δ	9.436	0.1001	9.229	9.642	94.27	<1.0E-37	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	337900	112600	3	13560	<1.0E-37	Significant
Lack of Fit	123.5	30.87	4	7.698	5.5E-04	Significant
Pure Error	84.21	4.01	21			
Residual	207.7	8.308	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance Test	15.03	12.59	0.0200	Unequal Variances
	Mod Levene Equality of Variance	1.991	2.573	0.1127	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9581	0.9264	0.3133	Normal Distribution
	Anderson-Darling A2 Normality Te	0.4918	2.492	0.2226	Normal Distribution

OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID: 13-5231-1319 **Endpoint:** 96h Cell Density **CETIS Version:** CETISv1.9.2
Analyzed: 20 Jul-17 16:02 **Analysis:** Nonlinear Regression (NLR) **Official Results:** Yes

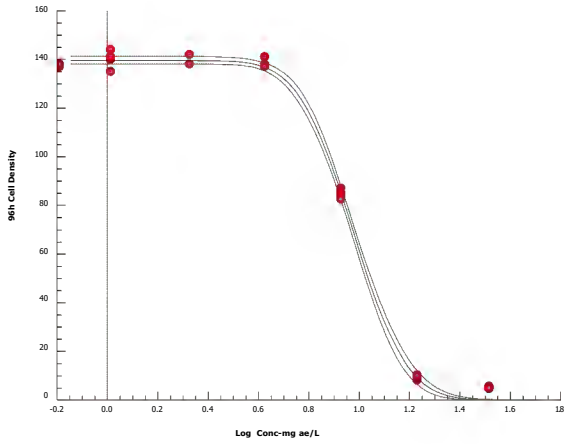
96h Cell Density Summary

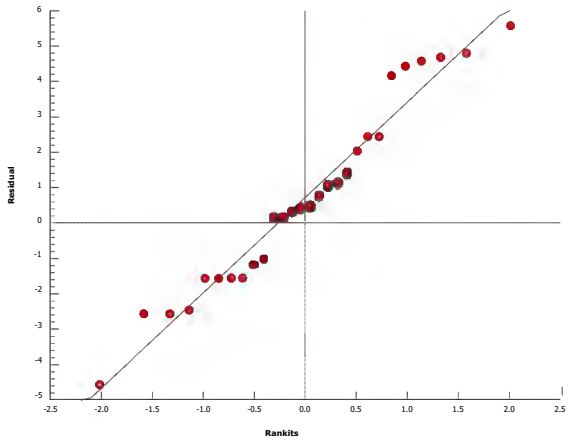
			Calculated Variate						
Conc-mg ae/L	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	137.5	137	138	0.2887	0.5774	0.42%	0.0%
1.03		4	140	135	144	1.871	3.742	2.67%	-1.82%
2.12		4	140	138	142	1.155	2.309	1.65%	-1.82%
4.21		4	138.2	137	141	0.9465	1.893	1.37%	-0.55%
8.47		4	84.65	82.5	87	0.97	1.94	2.29%	38.44%
17		4	9.555	8.22	10.3	0.4615	0.9231	9.66%	93.05%
32.8		4	5.003	4.67	5.67	0.227	0.454	9.08%	96.36%

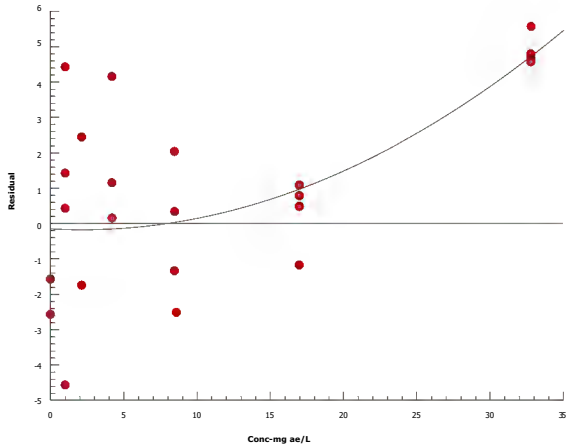
96h Cell Density Detail

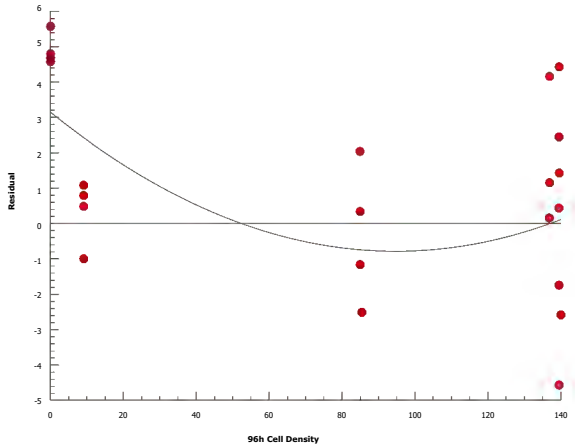
Conc-mg ae/L	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	137	138	137	138
1.03		140	135	144	141
2.12		138	138	142	142
4.21		138	137	141	137
8.47		87	85.3	83.8	82.5
17		10.3	8.22	9.7	10
32.8		4.78	5.67	4.67	4.89

Graphics Model: 3P Cum Log-Normal (Probit): μ=α·[1- Φ[log[x/δ]/γ]] Distribution: Normal [ω=1]









OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID:	09-7344-9992	**Endpoint:**	96h Growth Rate	**CETIS Version:**	CETISv1.9.2
Analyzed:	20 Jul-17 16:02	**Analysis:**	Nonlinear Regression (NLR)	**Official Results:**	Yes
Batch ID:	13-5501-2686	**Test Type:**	Algal Cell Growth (96-h)	**Analyst:**	
Start Date:	19 Oct-15	**Protocol:**	OCSPP 850.4500 Aquatic Plant (Algae)	**Diluent:**	Algal medium with silica
Ending Date:	23 Oct-15	**Species:**	Navicula pelliculosa	**Brine:**	
Duration:	96h	**Source:**	Lab In-House Culture	**Age:**	3d
Sample ID:	10-5408-5563	**Code:**	49833102	**Client:**	CDM Smith - K. Bozicevich
Sample Date:	19 Oct-15	**Material:**	2,4-DB	**Project:**	
Receipt Date:		**Source:**	2,4-DB Task Force		
Sample Age:	n/a	**Station:**			

Comments:

PC Code 030801, MRID 49833102
'96h AUC' endpoint...
As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

'96h Growth Rate' endpoint...
There are a total of 4 tied group(s) detected. CETIS applied a tie correction to the asymptotic normal approximation in cases where ties occurred across groups. Since Monte Carlo simulations can also effectively address ties, it is suggested that you re-analyze the data with Monte Carlo.
PC Code 030801, MRID 49833102

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Cum Log-Normal (Probit): μ=α·[1- Φ[log[x/δ]/γ]]	Normal [ω=1]	Off [μ*=μ]	None	None

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
11	75.79	-144.6	-141.6	0.9541	Yes	235.3	2.84	0.0000	Significant Lack of Fit

Point Estimates

Level	mg ae/L	95% LCL	95% UCL
IC5	4.469	3.078	5.56
IC10	6.222	4.938	7.401
IC25	10.82	9.484	12.19
IC50	19.99	18.26	21.88

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	1.253	0.02011	1.212	1.294	62.32	<1.0E-37	Significant Parameter
γ	0.9108	0.08195	0.742	1.08	11.11	<1.0E-37	Significant Parameter
δ	19.99	0.9839	17.96	22.02	20.32	<1.0E-37	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	31.37	10.46	3	2174	<1.0E-37	Significant
Lack of Fit	0.1176	0.02941	4	235.3	<1.0E-37	Significant
Pure Error	0.002625	0.000125	21			
Residual	0.1203	0.004811	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Mod Levene Equality of Variance	1.649	2.573	0.1832	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9105	0.9264	0.0203	Non-Normal Distribution
	Anderson-Darling A2 Normality Te	1.073	2.492	0.0083	Non-Normal Distribution

OCSPP 850.4500 Algal Toxicity **ABC Labs**

Analysis ID: 09-7344-9992 **Endpoint:** 96h Growth Rate **CETIS Version:** CETISv1.9.2
Analyzed: 20 Jul-17 16:02 **Analysis:** Nonlinear Regression (NLR) **Official Results:** Yes

96h Growth Rate Summary

			Calculated Variate						
Conc-mg ae/L	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
0	N	4	1.23	1.23	1.23	0	0	0.00%	0.0%
1.03		4	1.238	1.23	1.24	0.0025	0.005	0.40%	-0.61%
2.12		4	1.235	1.23	1.24	0.002886	0.005771	0.47%	-0.41%
4.21		4	1.233	1.23	1.24	0.002498	0.004995	0.41%	-0.2%
8.47		4	1.113	1.11	1.12	0.002499	0.004998	0.45%	9.55%
17		4	0.59	0.56	0.61	0.0108	0.0216	3.66%	52.03%
32.8		4	0.445	0.43	0.47	0.00866	0.01732	3.89%	63.82%

96h Growth Rate Detail

Conc-mg ae/L	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
0	N	1.23	1.23	1.23	1.23
1.03		1.24	1.23	1.24	1.24
2.12		1.23	1.23	1.24	1.24
4.21		1.23	1.23	1.24	1.23
8.47		1.12	1.11	1.11	1.11
17		0.61	0.56	0.59	0.6
32.8		0.44	0.47	0.43	0.44

Graphics Model: 3P Cum Log-Normal (Probit): μ=α·[1- Φ[log[x/δ]/γ]] Distribution: Normal [ω=1]